NUTRITION, FEEDING, AND CALVES

Modulation of Fat-Soluble Vitamin Concentrations and Blood Mononuclear Leukocyte Populations in Milk Replacer-Fed Calves by Dietary Vitamin A and β-Carotene[1]

B. J. NONNECKE,[2] R. L. HORST, W. R. WATERS,[3] P. DUBESKI,[4] and J. A. HARP
Metabolic Diseases and Immunology Research Unit,
USDA-ARS, National Animal Disease Center,
2300 Dayton Avenue,
Ames, IA 50010-0070

ABSTRACT

Dairy calves (n = 18), separated from dams at birth, were fed 1 L of pooled-colostrum. For the remaining 7 wk of the study, they were fed one of three diets consisting of either a custom-formulated milk replacer without vitamin A (controls), or supplemented with retinyl palmitate (equivalent to 32,000 IU of vitamin A/d) or with β-carotene (equivalent to 20,000 IU of vitamin A/d). Plasma retinol, β-carotene, and RRR-α-tocopherol concentrations were lowest at birth, and increased substantially from birth to 1 wk postpartum in all groups, a probable consequence of ingestion of colostrum. From 1 to 7 wk of age, retinol concentrations were greatest in retinyl palmitate-supplemented calves, intermediate in β-carotene-supplemented calves and lowest in control calves. At 2, 3, 5, 6, and 7 wk, RRR-α-tocopherol concentrations were lower in retinyl palmitate-supplemented calves than in control calves. A negative correlation between plasma retinol and vitamin E concentrations existed from wk 2 to 7, suggesting vitamin A influences the absorption and distribution of RRR-α-tocopherol. Supplemental retinyl palmitate, but not β-carotene, was associated with a reduction in the percentage of blood mononuclear leukocytes expressing CD2, CD4, and CD8 T cell antigens and interleukin-2 receptors. By wk 7, leukocyte populations from retinyl palmitate-supplemented calves were more similar to those from adult cattle than those from control calves, suggesting that supplemental vitamin A, as retinyl palmitate, affects the maturation of the neonatal immune system. Differences in the composition of blood mononuclear leukocyte populations may represent changes in immune competency.
(**Key words:** vitamin A status, vitamin E, neonatal calf, immune function)

Abbreviation key: BC = β-carotene, **HBSS** = Hank's balanced salts solution, **IL-2r** = interleukin-2 receptor, **MHC** = major histocompatibility, **MNL** = mononuclear leukocytes.**RRR-α-tocopherol** = d-alpha-tocopherol, the natural stereoisomer of vitamin E.

INTRODUCTION

In newborn calves, plasma concentrations of vitamin A (retinol, < 40 ng/ml) and the provitamin A compound, β-carotene (**BC**, < 4 ng/ml) are extremely low when compared to "normal" values for adult dairy cows (retinol, 400 to 700 ng/ml (35) and BC, > 500 ng/ml, unpublished data, R. L. Horst). In the United States, newborn calves are frequently fed milk replacers supplemented with vitamin A at concentrations that are more than 10-fold higher than the NRC requirement [14.1 μg of vitamin A/kg of live weight daily or approximately 1800 IU/d for a 40 kg calf (25)]. This practice is justified by the desire to provide formulated diets that ensure optimal growth and resistance to infectious disease.

Research supporting this practice, however, is limited. Results from a recent study (10) indicate that feeding milk replacers with high concentrations (87,000 IU of vitamin A/kg) of vitamin A does not affect the bioavailability of vitamin E and improves fecal consistency in young dairy calves. Other studies (8, 35, 38), however, suggest that high concentrations of dietary vitamin A decrease the bioavailability of vitamin E in young and adult dairy cattle. The potential for relatively high concentrations of vitamin A in commercial milk replacers to depress plasma vitamin E concentrations in young dairy calves (<7 wk of age) should be of concern because of the established role of vitamin E as a biologic antioxidant and free radical scavenger (21, 25).

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[2]To whom reprint request should be addressed.
[3]Present address: Veterinary Medical Research Institute, Iowa State University, Ames, IA 50011.
[4]Present address: Agriculture and Agri-Food Canada Research Center, Lacombe, AB, Canada.

The neonatal calf is extremely vulnerable to a variety of infectious diseases. The developmental immaturity of the neonatal immune system is considered contributory to the newborn's increased susceptibility to infectious disease (3). Peripheral blood mononuclear leukocytes (**MNL**) from 1-wk-old calves fed colostrum and milk are functionally hyporesponsive when compared to MNL populations from adult cows, even though they are equally capable of synthesizing DNA when stimulated with mitogen (30). The capacity of MNL from preruminant calves to produce inducible nitric oxide, a component of bactericidal mechanism of phagocytic leukocytes, and interferon-γ, a pivotal cytokine in the development of cell-mediated immunity, is also substantially different than the capacity of MNL from adult cows (30, 31). Peripheral blood MNL populations from calves also have higher proportions of CD4 T cells (30), CD8 T cells (30), $\gamma\delta$ T cells (20, 30), and monocytes (30), and lower proportions of B cells (5, 15) than do MNL populations from adult cattle. Vitamin A, by its conversion to retinoic acid, regulates genes which ultimately control the growth and differentiation of cells in a variety of tissues. In this role, vitamin A or BC, as a provitamin A compound, may promote the maturation of the neonatal immune system, allowing it to develop competency earlier when the calf is at greatest risk of infection.

The objective of the present study was to evaluate the effects of dietary vitamin A as retinyl palmitate or BC on the concentrations of retinol, BC, and vitamin E in plasma, and the composition of peripheral blood MNL populations of neonatal calves from birth to wk 7 of age.

MATERIALS AND METHODS

Calves and Diet

Eighteen male Jersey calves (average BW, 40 kg) from dams housed in the USDA-ARS, National Animal Disease Center (NADC) dairy facility and the Department of Animal Science Dairy Facility, Iowa State University, Ames, were obtained from May to November, 1993. Calves were birthed in clean pens, and removed to individual stalls shortly after birth. All calves were bottle-fed 1 L of pooled colostrum to assure adequate intake of γ-globulins for passive immunity, and then moved to an environmentally controlled isolation barn at the NADC to minimize exposure to infectious agents. For the duration of the study, calves were housed individually in hutches (1.5 m $\times$ 2.5 m) with raised decks made of fusion-coated wire mesh.

During the 7-wk study, calves were bottle-fed a custom-made milk replacer (Milk Specialties, Dundee, IL) at 10% of BW/d (divided between feedings at 0700 and 1500 h). Milk replacer was formulated to contain not less than 22% CP, not less than 20% fat, 40% lactose, and essential minerals. The milk replacer was not supplemented with esterified vitamin A during production; however, vitamin E (44.4 IU/kg), and vitamin D_3 (22.12 KIU/kg) were added.

Calves were divided randomly into groups (n = 6/group) and fed milk replacer only (low vitamin A diet), milk replacer with BC [50 mg/d or approximately 20,000 IU of vitamin A/d, given 1 mg of BC = 400 IU of vitamin A (25)] as a beadlet formulation containing 10% BC (Hoffmann-LaRoche, Inc., Nutley, NJ), or milk replacer with a water dispersible form of retinyl palmitate (36,000 IU of vitamin A/d, Hoffmann-LaRoche, Inc.). Supplements were refrigerated in the dark when not in use. Diets were reconstituted to a solution of approximately 1 part milk replacer to 8 parts warm, clean water immediately before feeding.

Calf-related protocols were approved by the Institutional Animal Care and Use Committee of the NADC.

Measurement of Fat-Soluble Vitamins in Plasma

Blood samples for determination of the concentrations of retinol, BC, and RRR-α-tocopherol in plasma were obtained by jugular venipuncture. The first sample was taken within 3 h of birth and before the calves had received colostrum. The 1-wk sample was taken on average 5.5 d after birth. All subsequent samples were taken at 7-d intervals. Plasma samples were protected from light and frozen at –80°C until analysis by reverse-phase HPLC, as described by Franklin et al. (12), using a modification of a method originally described by Kaplan et al. (23).

Phenotypic Analysis of Blood MNL Populations

Blood samples used for phenotypic analysis of circulating MNL populations were obtained by jugular venapuncture weekly from 1 to 7 wk of age. The 1st-wk sample was taken on average 5.5 $\pm$ 1.4 d after birth and at 7-d intervals thereafter. Calves were bled in the a.m. immediately before the first feeding of the day. Blood was collected into a 10% (vol/vol) solution of citric acid (0.083 *M*), sodium citrate dihydrate (0.153 *M*), and dextrose (0.244 *M*). Isolation and enrichment of MNL was by density gradient centrifugation as described previously (27). Contaminating erythrocytes were eliminated by hypotonic lysis prior to density gradient centrifugation of buffy coat cells.

The MNL-enriched populations were phenotyped by modifications of a flow cytometric procedure described previously (28). Briefly, enriched MNL were washed with and resuspended in cold Hank's balanced salts solution (**HBSS**) with 1% heat-inactivated fetal calf serum (FCS, Hyclone Laboratories, Inc., Logan, UT) and 0.1% NaN_3

TABLE 1. Primary antibodies used in the immunostaining of blood mononuclear leukocytes from control calves and calves supplemented with retinyl palmitate or β-carotene.

Antibody specificity[2]	Designation	Ig Isotype	Source[1]
CD2 T cell	BAQ95A	IgG_1	VMRD
CD4 T cell	GC50A1	IgM	VMRD
CD8 T cell	CACT80C	IgG_1	VMRD
B cell	BAQ44A	IgM	VMRD
Monocyte	IL-A46	IgM	ILRAD
γδ T cell	IL-A29	IgG_1	ILRAD
IL-2r	CACT116A	IgG_1	VMRD
MHC class II	TH14B	IgG_2	VMRD

[1]VMRD = VMRD, Inc. (Pullman, WA), ILRAD = International Laboratory for Research in Animal Diseases (Nairobi, Kenya).

[2]IL-2r = Interleukin-2 receptor, MHC = major histocompatibility complex.

at a density of 10×10^6 leukocytes/ml. Approximately 2×10^5 leukocytes were added to individual wells of a 96-well U-bottom microtiter plate. Monoclonal antibodies (Table 1), diluted in HBSS with 1% FCS and 0.1% NaN_3 were added (50-μl aliquots) individually to the wells. Plates were incubated at 4°C for 15 min and were subsequently washed twice by centrifugation ($1171 \times g$ at 4°C for 2 min). Supernatants were removed with a plate washer (Dynatech Miniwash; Dynatech Laboratories, Alexandria, VA) and cells were resuspended in HBSS with 0.1% NaN_3 and incubated (4°C for 15 min) with the second antibody [fluorescein isothiocyanate-conjugated goat $F(ab')_2$ fragments against mouse IgG or IgM, 50 μl; Organon-Teknika-Cappel, Durham, NC]. Plates were then washed and cell pellets were resuspended in HBSS with 0.1% NaN_3. Nonspecific binding of antibody was assessed by incubating each test sample with secondary antibody alone.

A FACScan® (Becton Dickinson Immunocytometry Systems, San Jose, CA) was used for flow cytometric analyses of 5000 cells that exhibited light scattering properties consistent with MNL from dairy cattle. An argon laser with an excitation wavelength of 488 nm was used to detect cells associated with fluorescein isothiocyanate-conjugated secondary antibodies. Emission fluorescence was detected with a 530-nm bandpass filter and converted to $\log_{10}$ fluorescence. Markers were positioned for negative control samples to provide a background of ~2% and were maintained at this position for all samples. Cells with fluorescence intensities greater than the negative control were considered positive. The fluorescence data associated with each parameter were expressed as a percentage of the gated MNL population.

Statistical Analysis

Data were assessed for normality, and the majority of the data were $\log_{10}$-transformed prior to statistical analysis. Data were analyzed by a split-plot repeated measures ANOVA (StatView, SAS Institute, Inc., Cary, NC). The statistical model included effects of dietary treatment, day, and interaction of day and treatment. Where statistical differences ($P < 0.05$) were detected, the Tukey Kramer multiple-comparison test was applied.

Pearson's product-moment correlations were computed between mean concentrations of vitamins (retinol and α-tocopherol) and percentages of leukocytes expressing specific surface antigens.

For the purpose of data presentation, all data are presented as arithmetic means (±SEM).

RESULTS

Plasma Concentrations of Retinol, β-Carotene, and Vitamin E

Retinol concentrations in the plasma of control and supplemented calves were not different ($P = 0.25$) at birth and averaged 29.1 ng/ml (n = 18, Figure 1a). Retinol concentrations in 1-wk-old control calves (95.6 ng/ml), and BC-supplemented (109.0 ng/ml) and retinyl palmitate-supplemented calves (157.6 ng/ml) were higher ($P < 0.01$) than those of newborns (Figure 1a). Retinol concentrations did not change from wk 1 to 7 in control ($P = 0.71$), BC-supplemented ($P = 0.07$), and retinyl palmitate-supplemented ($P = 0.99$) calves. At wk 1, 3, 4, 6, and 7, plasma retinol concentrations in calves supplemented with retinyl palmitate, were higher ($P < 0.05$) than the corresponding concentrations in control calves. Retinol concentrations in BC-supplemented calves were not different statistically from concentrations in unsupplemented control calves from wk 1 to 7 (Figure 1a).

β-Carotene concentrations in the plasma of control and supplemented calves were not different ($P = 0.34$) at birth and averaged 0.4 ng/ml (n = 18, Figure 1b). Because differences between BC concentrations in BC-supplemented calves and calves in the other groups were large from wk 1 to 7, the ordinate of Figure 1b employs a logarithmic scale. β-Carotene concentrations in 1-wk-old control (34.4 ng/ml) and retinyl palmitate-supplemented calves (52.8 ng/ml) were higher ($P < 0.001$) than values at birth (Figure 1b). At wk 1, BC-supplemented calves had much higher ($P < 0.0001$) BC concentrations (1172 ng/ml) than calves in the other groups. β-Carotene concentrations in control calves remained unchanged ($P = 0.53$) from wk 1 to 7. In retinyl palmitate-supplemented calves, BC concentrations decreased ($P = 0.02$) from wk 1 to 7, reaching 24.0 ng/ml by wk 7. β-Carotene concentrations in BC-supplemented calves increased ($P = 0.0002$) progressively during this period, reaching 2451 ng/ml by wk 7.

Concentrations of RRR-α-tocopherol in the plasma of control and supplemented calves were not different (P = 0.15) at birth and averaged 119.4 ng/ml (n = 18, Figure 1c). At wk 1 postpartum, RRR-α-tocopherol plasma concentrations in control (1171 ng/ml), BC-supplemented (1154 ng/ml) and retinyl palmitate-supplemented calves (1357 ng/ml) were not different but were higher (P < 0.001) than at birth (Figure 1c). RRR-α-Tocopherol levels in retinyl palmitate-supplemented calves remained unchanged (P = 0.78) from wk 1 to 7. In contrast, concentrations in control and BC-supplemented calves increased (P < 0.05) from wk 1 to 2 and at wk 2 were higher (P < 0.05) than those in retinyl palmitate-supplemented calves. In unsupplemented calves, RRR-α-tocopherol plasma concentrations remained elevated for the duration of the study and were higher (P < 0.05) than those in retinyl palmitate-supplemented calves at wk 2, 3, 5, 6, and 7. During this period, the concentrations of RRR-α-tocopherol in BC-supplemented calves decreased (P = 0.007) and by wk 7 were not different from those in retinyl palmitate-supplemented calves. Even though the RRR-α-tocopherol concentrations in retinyl palmitate-supplemented group remained low throughout the experimental period, these calves did not manifest symptoms (i.e., muscular and dystrophic lesions) of vitamin E deficiency.

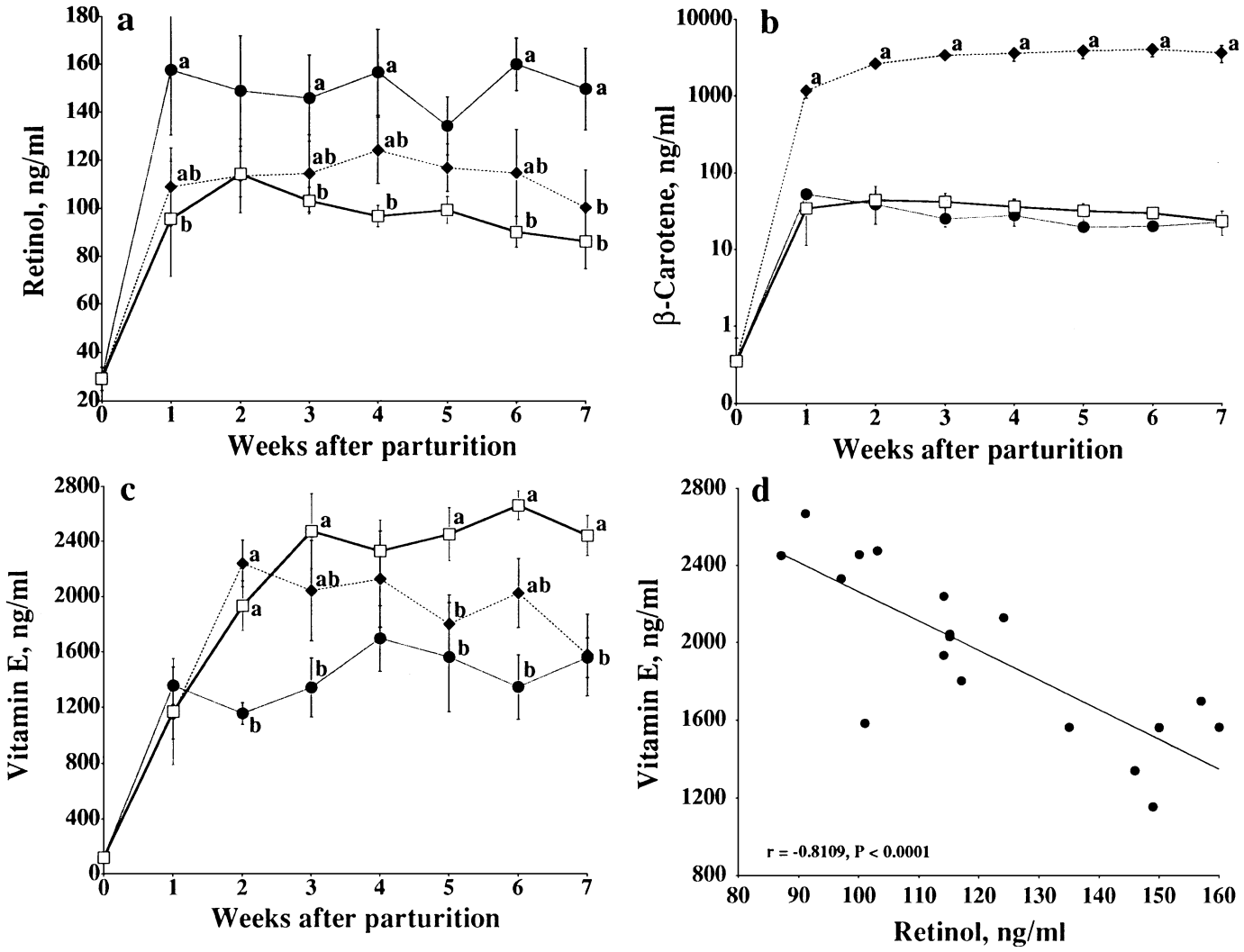

Figure 1. Concentrations (mean ± SEM) of retinol (a), β-carotene (b), and RRR-α-tocopherol (c) in plasma from control calves (□) and calves supplemented orally with retinyl palmitate (●) or β-carotene (◆). The relationship between the mean plasma concentrations (ng/mL) of retinol and vitamin E (RRR-α-tocopherol) is shown in d; values are weekly treatment means for plasma concentrations of vitamin E and retinol in plasma of control and supplemented groups of calves from wk 2 to 7 (n = 18). At birth, concentrations of retinol, β-carotene, and RRR-α-tocopherol were less (P < 0.0001) than all subsequent values. Week 1 on the abscissa represents samples taken on average 5.5 ± 1.4 d after birth. All subsequent samples were taken at 7-d intervals. Letters indicate means that differed (P < 0.05) at specific time.

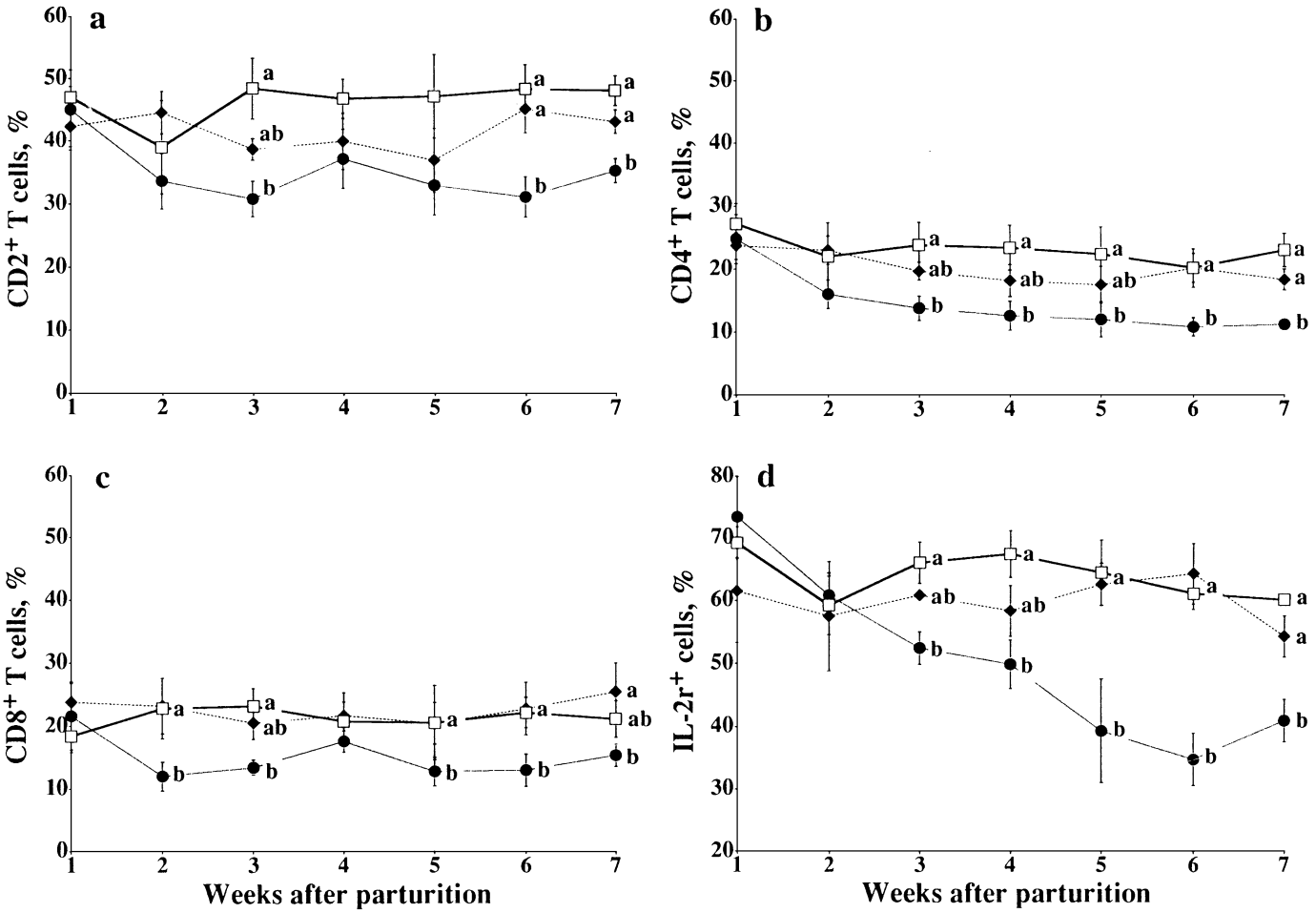

Figure 2. Percentages (mean ± SEM) of CD2 (a), CD4 (b), CD8 (c), and interleukin-2 receptor (IL-2r)-positive cells (d) in blood mononuclear leukocyte populations from control calves (□), and calves supplemented orally with retinyl palmitate (●) or β-carotene (◆). Week 1 on the abscissa represents samples taken on average 5.5 ± 1.4 d after birth. All subsequent samples were taken at 7-d intervals. Letters indicate means that differed ($P < 0.05$) at a specific time.

Data summarized in Figure 1a and c suggested an inverse relationship between the mean weekly treatment concentrations of retinol and vitamin E in all 2- to 7-wk-old calves. The Pearson's product-moment correlation between the mean plasma concentrations of retinol and RRR-α-tocopherol in all calves during this period was strongly negative (r = –0.8109; $P < 0.0001$). This relationship is shown in Figure 1d.

Composition of Blood MNL Populations

Results from flow cytometric analysis of peripheral blood MNL populations from control and supplemented calves are summarized in Figures 2, 3, and 4. Treatment-specific effects on the composition of MNL populations [CD2 cells, $P = 0.70$; CD4 cells, $P = 0.72$; CD8 cells, $P = 0.61$; interleukin-2 receptor (**IL-2r**)-positive cells, $P = 0.18$; B cells, $P = 0.99$; major histocompatibility (**MHC**); class II cells, $P = 0.65$; and γδ cells, $P = 0.48$] were not evident at wk 1. Leukocyte populations in BC-supplemented calves were not different (CD2 cells, $P = 0.06$; CD4 cells, $P = 0.08$; CD8 cells, $P = 0.99$; IL-2r-positive cells, $P = 0.18$; B cells, $P = 0.73$; MHC class II cells, $P = 0.56$; and γδ cells, $P = 0.20$) from those in control calves at any time during the experimental period.

Percentages of CD2, CD4, and CD8 T cells and IL-2r-positive leukocytes (Figure 2a to d) in MNL populations from retinyl palmitate-supplemented calves were different ($P < 0.05$) from those in unsupplemented controls. Percentages of CD2 T cells in retinyl palmitate-supplemented calves decreased ($P = 0.06$) from 45.4% (wk 1) to 35.4% (wk 7) (Figure 2a), and were lower than those in control calves at wk 3, 6, and 7. Percentages in control and BC-supplemented calves remained unchanged (controls: $P = 0.88$; BC group: $P = 0.41$) from wk 1 to 7.

Percentages of CD4 T cells in MNL populations from calves fed retinyl palmitate declined ($P = 0.05$) from 24.8% (wk 1) to 11.2% (wk 7), and were lower ($P < 0.05$) than the corresponding percentages in control calves from wk 3 to 7 (Figure 2b). In contrast, CD4 T cell percentages in control ($P = 0.88$) and BC-supplemented ($P = 0.41$) calves did not change from wk 1 to 7.

Percentages of CD8 T cells in retinyl palmitate-supplemented calves decreased ($P = 0.0814$) from 21.6% (wk 1) to 15.4% (wk 7), and at wk 2, 3, 5, 6, and 7 were lower ($P < 0.05$) than the corresponding percentages in control calves (Figure 2c). The CD8 T cell percentages in control ($P = 0.68$) and BC-supplemented ($P = 0.96$) calves did not change from wk 1 to 7.

Percentages of IL-2r positive cells in MNL populations from retinyl palmitate-supplemented calves declined ($P = 0.0007$) from 73.4% (wk 1) to 40.8% (wk 7), and were lower ($P < 0.05$) than the corresponding values for control calves at wk 3, 4, 5, 6, and 7 (Figure 2d). In contrast, percentages of IL-2r positive leukocytes in unsupplemented ($P = 0.37$) and BC-supplemented calves ($P = 0.90$) did not change from 1 to 7 wk of age.

The Pearson's product-moment correlations between plasma retinol concentrations and percentage of MNL phenotypes in all calves from wk 2 to 7 were negative for plasma concentrations of retinol and the percentages of CD2 ($r = -0.8856$; $P < 0.0001$), CD4 ($r = -0.8758$; $P < 0.0001$), CD8 ($r = -0.7854$; $P < 0.0001$) , and IL-2r (r =

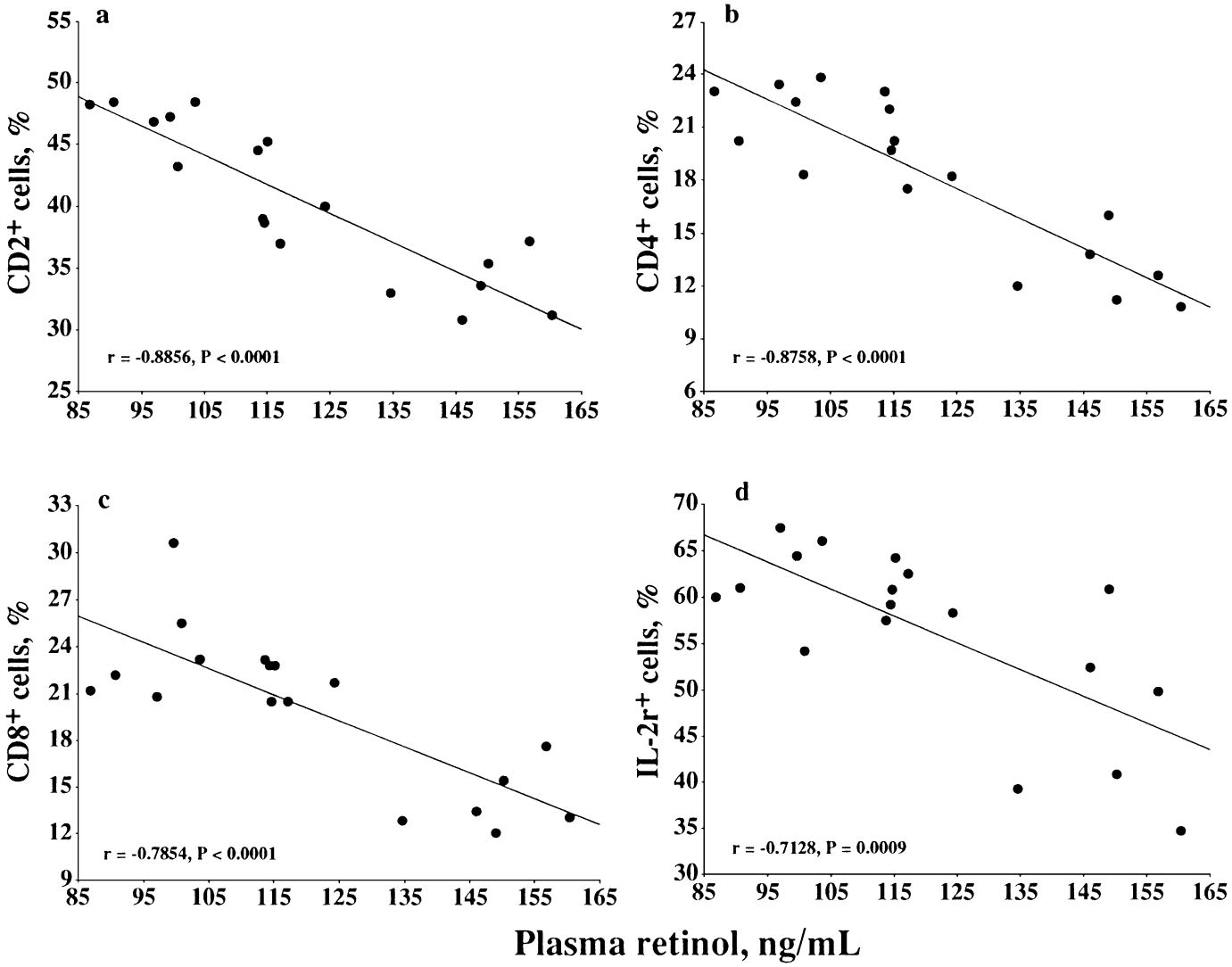

Figure 3. Correlations between the plasma concentrations of retinol and the percentage of CD2 T cells (a), CD4 T cells (b), CD8 T cells (c), and interleukin-2 receptor (IL-2r)-positive cells (d) in peripheral blood of calves. Values correspond to weekly treatment mean retinol concentrations and leukocyte percentages for control and supplemented groups of calves from wk 2 to 7 (n = 18).

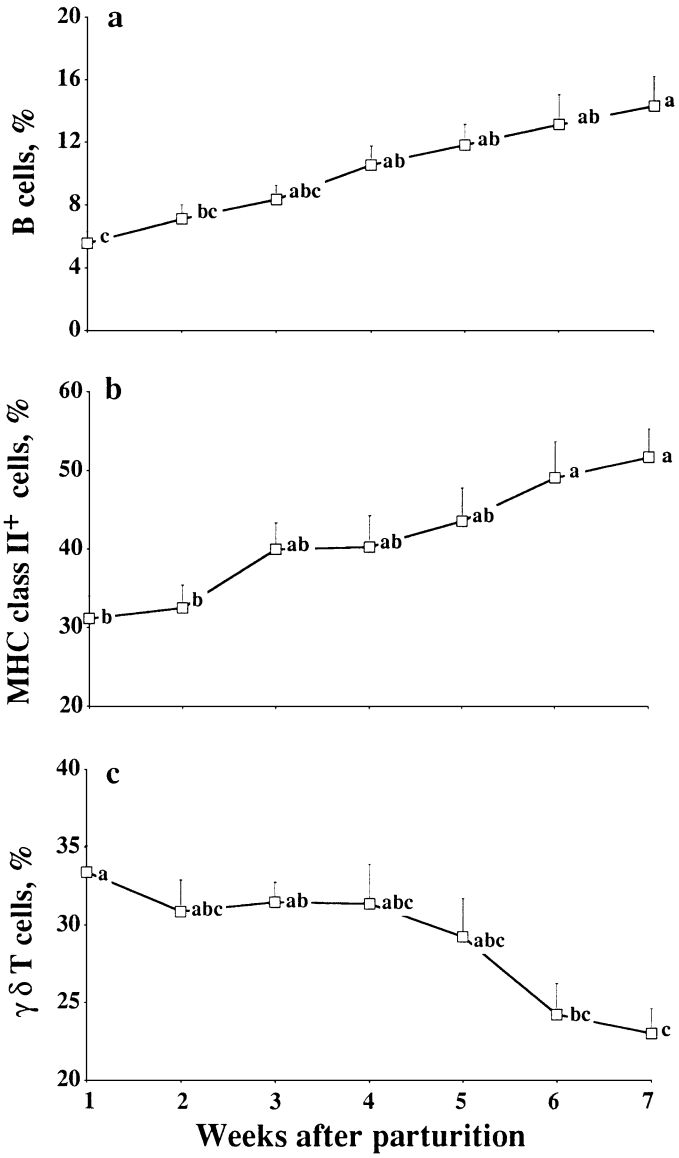

Figure 4. Percentage (mean ± SEM) of B cells (a), major histocompatibility class (MHC) II+ cells (b) and $\gamma\delta$ T cells (c) in peripheral blood of all calves (n = 18). Week 1 on the abscissa represents samples taken on average 5.5 ± 1.4 d after birth. All subsequent samples were taken at 7-d intervals. Letters indicate means that differed ($P < 0.05$) at a specific time.

–0.7128; $P = 0.0009$) positive cells in peripheral blood MNL populations. These relationships are shown graphically in Figure 3.

Although percentages of B cells, major histocompatibility (**MHC**) class II+ cells, and $\gamma\delta$ T cells in blood MNL populations were unaffected by treatments, they were influenced by age (Figure 4). The proportion of B cells increased ($P < 0.0001$, n = 18) progressively from 5.6% (wk 1) to 14.3% (wk 7) (Figure 4a). Percentages of MHC class II+ cells also increased ($P = 0.0006$, n = 18) from 31.1% (wk 1) to 51.7% (wk 7) (Figure 4b). In contrast, the proportion of $\gamma\delta$ T cells in the MNL population decreased ($P = 0.001$, n = 18) from 33.4% (wk 1) to 23.1% (wk 7) (Figure 4c). Monocyte percentages were unaffected ($P = 0.42$) by age (data not shown) and during the 7-wk period ranged from 17.1% (wk 1) to 20.2% (wk 3).

DISCUSSION

Plasma Vitamin Concentrations

Of the 24 calves entered into the study, 6 were excluded because they developed significant health problems (i.e., scours) shortly after entering the study. These problems were not associated with any specific treatment. The remaining healthy calves (n = 18) were the source of the data collected in this study. Their health during the 7-wk study was optimized by housing them in individual hutches located in clean, environmentally-controlled rooms maintained at a uniform temperature of approximately 20°C. The use of isolation rooms and a low vitamin A diet allowed the effects of dietary vitamin A and BC to be evaluated under controlled experimental conditions.

Plasma concentrations of retinol, BC, and vitamin E at birth were extremely low relative to those in adult cattle (21, 25, 30) and those in the same calves at wk 1 of age. The pronounced increases in the concentrations of these vitamins in all calves during the first week was likely caused, in part, to the ingestion of colostrum that was provided to assure adequate intake of γ-globulins. Rajaraman et al. (30) showed recently that feeding skimmed versus whole colostrum and milk to newborn calves (≤ 7 d of age) does not affect acquisition of passive immunity, but does attenuate the increase in plasma retinol, BC, and RRR-α-tocopherol concentrations associated with the ingestion of whole colostrum. In retrospect, feeding skimmed rather than whole colostrum might have produced greater segregation of control and treatment groups, based on plasma retinol and BC concentrations, without compromising passive immunity.

Dietary vitamin A, as retinyl palmitate, at a concentration comparable to that present in commercial milk replacers caused only a modest increase in plasma concentrations of retinol. These results indicate that the concentration of retinol in plasma is not related solely to the amount of vitamin A provided in the diet and suggest that plasma concentration of retinol may not be a reliable indicator of vitamin A status. The maximum concentration of retinol in the plasma of retinyl acetate-supplemented calves was somewhat lower than the 200 ng/ml considered suggestive of a vitamin A deficiency in growing calves (9, 25). In unsupplemented calves, retinol concentrations were frequently <100 ng/ml, indicating a more advanced stage of deficiency (9, 25). Vitamin A

status of neonatal calves was evaluated recently with a relative dose response assay in conjunction with analyses of retinol concentrations in plasma and liver tissue (19). Comparing results from these assays, the authors concluded that plasma retinol concentrations substantially <200 ng/ml are frequently not indicative of a vitamin A deficiency in the calf. The relative dose response assay and analysis of hepatic vitamin A, although providing a more accurate indication of vitamin A status, are too complex or invasive to be useful on the farm. In spite of the limitations of plasma retinol as a measure of vitamin A status, it continues to be the predominant standard for estimating vitamin A status of cattle and other species.

Dietary vitamin A, at approximately 20 times the recommended daily requirement (25), depressed vitamin E concentrations in the plasma of calves in the present study. There was also a negative association between the plasma retinol and RRR-α-tocopherol concentrations in all calves from wk 2 to 7, suggesting that a general, inverse relationship exists between the plasma concentrations of these fat-soluble vitamins in young, growing calves. Although Eicher et al. (10) failed to find any effect of high dietary concentrations of vitamin A on the concentrations of RRR-α-tocopherol in the plasma of calves fed milk replacers from d 3 to 45, others have shown that supplementing older calves, 2 to 6 mo of age, with more vitamin A depresses plasma vitamin E concentrations (17, 38). Franklin et al. (11) recently demonstrated that plasma vitamin E concentrations were depressed in newborn, milk-fed calves supplemented with 15,000 or 30,000 IU of vitamin A/d. Similar relationships between vitamins A and E have been observed in other species (6, 7, 22). Because of the importance of vitamin E as a biologic antioxidant and free radical scavenger (18) and mounting evidence indicating its beneficial effects on the immune system of the calf (22, 32), the impact of dietary vitamin A on the bioavailability of vitamin E in the neonatal calf warrants further research. Providing additional vitamin E to young calves may be effective in reducing or preventing the depression in plasma vitamin E. This approach has been shown to minimize the clearance of vitamin E in chicks fed high vitamin A diets (37); however, excess dietary vitamin E can adversely affect plasma and liver concentrations of vitamin A (1).

Mechanisms underlying the depression in plasma RRR-α-tocopherol associated with excess dietary vitamin A were not addressed in the present study and, in general, are poorly characterized. Intravenous administration of vitamin A, interestingly, appears to have no effect on circulating concentrations of vitamin E in rats (17) and chicks (13). Dietary vitamin A may antagonize intestinal absorption of vitamin E specifically (6) and the intestinal absorption of vitamin E is less efficient than that of vitamin A and retinoids (14). Based on this hypothesis, the high concentrations of retinyl palmitate and substantially lower concentrations of vitamin E in the diet used in the present study may have reduced the uptake of vitamin E, depressing RRR-α-tocopherol concentrations in the plasma. Elevated dietary vitamin A also promotes increased oxidation of dietary RRR-α-tocopherol in the intestinal tract (37).

In the present study, calves were fed a milk replacer devoid of exogenous vitamin A in an attempt to limit the amount of vitamin A in the basal diet. Vitamin A deficiency in dogs, induced by intestinal resection or by feeding a vitamin A-deficient diet, was associated with a pronounced increase in plasma vitamin E concentrations (16). This would suggest that the increased concentrations of RRR-α-tocopherol in the plasma of control calves may have been a consequence of a vitamin A deficiency induced by feeding a 'low vitamin A' diet. Subsequent analysis of low vitamin A milk replacers, similar to the one used in the present study, indicated that they frequently contain enough endogenous vitamin A to meet the NRC-recommended daily requirement (B. I. Nonnecke and S. T. Franklin, 1998, unpublished data). If so, control calves in the present study were not deficient in vitamin A and the sustained low concentrations RRR-α-tocopherol in the plasma of retinyl palmitate-supplemented calves may have been a consequence of excess dietary vitamin A.

The impact of supplemental BC on plasma retinol, BC, and RRR-α-tocopherol concentrations was also evaluated. The rapid and large increase in the concentrations of BC in the plasma of BC-supplemented calves during the first week supports previous research indicating that BC is rapidly absorbed by liquid-fed, preruminant calves (2). Although the supplemental BC was equivalent to 20,000 IU of vitamin A/d [1 mg of BC = 400 IU of vitamin A, (25)], retinol concentrations in BC-supplemented calves did not statistically exceed those in unsupplemented control calves at any time during the study. Our results and those from studies in humans (24, 26) indicate that sustained, daily supplementation with high concentrations of BC, although causing an increase in BC concentrations, has minimal impact on plasma retinol concentrations. Chew et al. (5) have also shown that oral administration of BC to calves fed a pelleted diet containing normal concentrations of vitamin A does not affect plasma retinol concentrations.

Leukocyte Subsets

Numerous studies in animals and humans have provided experimental and observational data indicating a strong association between vitamin A status, immune

function, and resistance to experimental and natural infections (reviewed in 33). Results from the present study indicate that supplemental vitamin A, as retinyl palmitate, may influence the composition of the blood MNL populations in young, growing calves fed milk replacer. Leukocyte subsets affected were those essential in the recognition of and response to antigen, and included T cell subsets expressing CD2, CD4, and CD8 cell-surface molecules. In addition, the percentage of cells expressing IL-2r, an activation antigen, was affected by dietary retinyl palmitate. The proportional contribution of these subsets decreased with age in retinyl palmitate-supplemented calves and remained unchanged in control calves. Dietary BC did not influence the composition of MNL populations. Results from studies evaluating the effects of supplemental BC on circulating leukocyte subsets in other species are somewhat inconsistent, indicating no effect (34) or a significant effect (reviewed in 4). It is evident from these reports that the experimental outcome is influenced by the age and species of subjects used in trials.

When associations between plasma retinol concentrations and the percentage contribution of leukocyte subsets to MNL populations were examined in all calves, negative correlations between plasma retinol concentration and the percentages of CD2, CD4, and CD8 T cells, as well as IL-2r positive cells, were found. Because this is a new observation, additional research is necessary to confirm the apparent relationship between vitamin A status and leukocyte subsets in the blood of the bovine neonate, and to determine whether the competency of the immune system is influenced by the effects of vitamin A on these subsets.

The percentages of CD2 (35.4%), CD4 (11.2%), and CD8 (15.4%) T cells, and IL-2r-positive cells (40.8%) in MNL populations from retinyl palmitate-supplemented calves at 7 wk of age were comparable to ranges of the percentages of these subsets in adult dairy cows (i.e., CD2 cells: 34 to 38%; CD4 cells: 14 to 17%; CD8 cells: 13 to 17%; IL-2r$^+$ cells: 32 to 38%) (12). These data suggest that dietary vitamin A may accelerate maturation of the adaptive arm of the neonatal calf's immune system. Additional experiments are necessary to determine whether the adult-like phenotype of the mononuclear population in the vitamin A-supplemented calves is associated with changes in immune function and infectious disease resistance.

Although the functional capacities of these leukocyte populations were not evaluated, vitamin A-induced changes in leukocyte subsets may have affected the functional capacity of the neonate's immune system. This possibility is given credence by a recent study (29) with young Holstein bulls demonstrating a positive correlation ($P < 0.05$) between CD4 and CD8 T cell percentages in blood MNL populations and the capacity of this population to secrete interferon-γ and immunoglobulin.

Vitamin A-induced compositional changes in MNL populations from young calves have not been reported previously. A recent study (11) examining the effects of dietary vitamin A (retinyl acetate at 0, 15,000, and 30,000 IU/d) on immune parameters of milk-fed neonatal calves from birth to 6 wk of age did not demonstrate an effect of vitamin A on percentages of CD2 T cells in the peripheral blood MNL populations. The apparent discrepancy between the results of these studies may have been caused by differences in dietary regimen. In the study by Franklin et al. (11), control and treated calves were fed a 'high vitamin A' diet consisting of colostrum (4.32 kg at birth) and milk (4.54 kg/d) plus a standard calf ration containing 9790 IU of vitamin A/kg of DM . The failure of the supplemental vitamin A (i.e., retinyl acetate) at 30,000 IU/d to influence plasma retinol concentrations in their study indicates that unsupplemented calves were likely vitamin A replete. In contrast, retinol concentrations in the plasma of control calves fed a low vitamin A diet in the present study were substantially lower than those in retinyl palmitate-supplemented calves from wk 1 to 7 postpartum. Studies in other species suggest that vitamin A status can influence leukocyte subsets in the peripheral circulation and that these changes are frequently associated with changes in their functional capacities (reviewed in 33). As with trials evaluating effects of BC, the outcome of these studies appears to be dependent on the species and age of animal used.

Percentages of B cells, $\gamma\delta$ T cells, monocytes, and MHC class II-positive leukocytes were not affected by dietary supplementation with either BC or retinyl palmitate. Age-related changes in B cell and $\gamma\delta$ T cell populations seen in the present study agree with those of previous studies indicating that proportions of B cells increase with age and the proportion of $\gamma\delta$ T cells decrease with age (11, 20, 30, 36). The increase in the percentage of MHC class II$^+$ cells with age has been reported previously (15). The longitudinal changes in leukocyte subsets, seen in all calves, likely reflect the maturation of the calf's immune system.

CONCLUSIONS

Results from the present study suggest the amount of vitamin A in the diet of calves fed milk replacer can alter the bioavailability of vitamin E and the composition of the peripheral blood mononuclear leukocyte population. Additional research is necessary to confirm these results and, secondly, determine whether these metabolic and immunologic changes influence the growth and health of the calf.

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